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Soil organic carbon stabilization mechanisms in a subtropical mangrove and salt marsh ecosystems

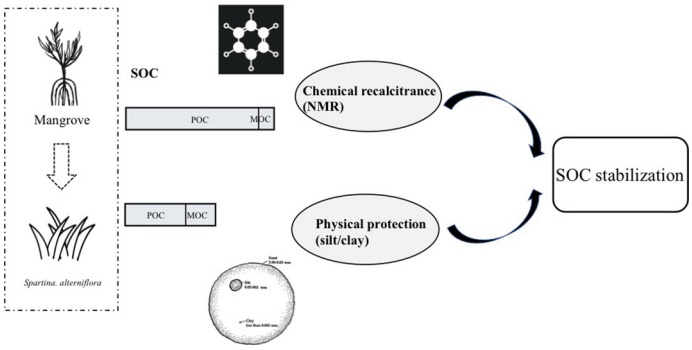
Huimin Sun [a], Jiang Jiang [a,*], Lina Cui [a], Wenting Feng [b,c], Yugang Wang [b], Jinchi Zhang [a]

[a] Collaborative Innovation Center of Sustainable Forestry in Southern China of Jiangsu Province, Nanjing Forestry University, Nanjing 210037, China
[b] State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Xinjiang 830011, China
[c] National Engineering Laboratory for Improving Quality of Arable Land, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

HIGHLIGHTS

- Stabilization mechanisms of SOC in mangroves and salt marshes are studied.
- SOC decomposition increased with *Spartina alterniflora* encroachment.
- Organo-mineral association plays a major role in salt marsh SOC stabilization.
- Recalcitrant C in mangrove soil contributes to SOC stabilization.
- Different SOC functional groups were associated with distinct microbial communities.

GRAPHICAL ABSTRACT



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ABSTRACT

Mangrove and salt marsh ecosystems are one of the most productive ecosystems in terrestrial ecosystems, playing an important role in global carbon (C) cycling. The anaerobic condition in coastal wetland usually impedes the decomposition of soil organic carbon (SOC). However, the intrinsic stabilization mechanisms of SOC other than environmental factors are poorly understood in coastal wetland. In this paper, we investigated the relative contribution of mineral association and chemical compounds in maintaining the stabilization of SOC in the mangrove/salt marsh ecotone, and how the microbial community is involved in the stabilization. From NMR spectroscopy, we found that the SOC molecular structure of *Spartina. alterniflora* soils is simpler than that in mangrove forest, indicating an increased SOC decomposition with invasion of *S. alterniflora*. On the contrary, the molecular structure of SOC in mangrove forest was dominated by recalcitrant aromatic C. Meanwhile, the larger fractions of silt/clay content in *S. alterniflora* and the transitional community were corresponding to higher percentage of mineral organic carbon (MOC), which suggest that the SOC in *S. alterniflora* vegetated soil was mainly protected by the mineral association. The transitional community contained highest MOC content probably due to both physical protection of mineral association and recalcitrant C input from adjacent mangroves. We also found that the fraction of SOC and its chemical structure of functional groups were associated with microbial communities. This study revealed the occurrence of different SOC stabilization mechanisms between mangroves and salt marshes. The knowledge gained may help to make predictions about future SOC dynamics as the different stabilization processes may response to climate change or human activities differently.

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* Corresponding author.
E-mail address: jiangjiang@njfu.edu.cn (J. Jiang).

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1. Introduction

Mangroves and salt marshes located along tropical to subtropical coastlines, play roles of significant importance in maintaining high productivity and biodiversity and providing a stable habitat for living things (Alongi, 2002; Donato et al., 2011). Although they cover only a small percentage of terrestrial coastal areas, mangroves and salt marshes have high C sequestration rates, and usually maintain soil C stocks that are typically greater than other terrestrial ecosystems (Bouillon et al., 2008; Alongi, 2014; Hutchison et al., 2014). As climate changes and sea level rises, many studies have been conducted to quantify changes of soil C stock due to vegetation shifts between mangroves and salt marshes (Comeaux et al., 2012; Bianchi et al., 2013; Doughty et al., 2016). Less attention, however, has been paid to understand the mechanisms of soil C stabilization in mangroves and salt marshes ecosystems, with regards to how organic C is preserved in soil.

The salt marshes normally are distributed in subtropical to temperate region whereas mangroves are located in subtropical or tropical zones. Therefore, the ecosystems of the two types of plants overlap in space and form ecotonal communities at subtropical intertidal zones, which are sensitive to climate change and sea level rise. Encroachment of mangroves into salt marshes has been observed on all continents (Saintilan et al., 2014) which could result in substantial increases in aboveground biomass but various effects on belowground soil C stocks. It has been found that encroachment of mangroves either cause an increase (Osland et al., 2012; Kelleway et al., 2016; Pérez et al., 2017) or have little effect on soil C (Perry and Mendelssohn, 2009; Henry and Twilley, 2013; Doughty et al., 2016). The different responses of salt marshes soil C to the encroachment of mangrove may derive from as the follows: 1) variation in precipitation pattern that controls the productivity of the salt marsh ecosystem that occupied the habitat previously (Yando et al., 2016), or 2) temporal lags in the effects of mangrove expansion, that accumulation of soil C takes decades after mangrove expansion (Perry and Mendelssohn, 2009; Henry and Twilley, 2013). The large aboveground biomass of mangrove forest should indeed increase the C input to coastal sediment (Doughty et al., 2016). But the dynamics of soil C stock also depends on stabilization of SOC. SOC stabilization has been demonstrated playing an important role in grasslands and forests as a mechanism to increase C sequestration (Wang et al., 2015; Cao et al., 2016; Luo et al., 2017; Shiau et al., 2017; Sarker et al., 2018), but to date, the mechanism is understudied in coastal ecosystems.

Over the past few decades, the stabilization mechanisms of SOC have been largely discussed with respect to physical protection by aggregates associated with minerals, and the nature of recalcitrant compounds of SOC (Cui et al., 2014; Throckmorton et al., 2015; Sarker et al., 2018; Wu et al., 2018). Increasing evidence suggests that physical protection plays more important role in the preservation of SOC and chemical make-up is less important (Almendros and González-Vila, 1987; Ekschmitt et al., 2008; Kleber et al., 2011; Guo et al., 2018). Indeed, organic matter with recalcitrant compounds are decomposable at the time scale of years to decades, which is much shorter than the residence time of most SOC (Fontaine et al., 2007; Schmidt et al., 2011). Furthermore, labile C can be occluded by microaggregates or adsorbed to silt/clay mineral surface, which restrict accessibility of microbes or extracellular enzymes to SOC (Six et al., 2002; Six and Jastrow, 2002; John et al., 2005). However, most of these conclusions were based on grassland or forest ecosystems. Comparison of the stabilization mechanisms is less studied in wetlands, where the SOC decomposition is limited by virous environmental factors such as anaerobic conditions (Davidson and Janssens, 2006).

Wetlands usually contain large amount of soil C due to the low oxygen levels, which limit decomposition of SOC (Chmura et al., 2003; Donato et al., 2011; Kirwan and Megonigal, 2013). Coastal wetlands occupy the intertidal zone where soils can be exposed to air at low tide and thus might be expected to have higher rates of SOC decomposition than permanently flooded wetlands (Temmerman et al., 2003; Marion et al., 2009). In addition to the environmental factors, soil properties may play a significant role in protecting SOC from decomposition. For example, clay content has found to be an important factor in controlling SOC dynamics in salt marshes (Lehmann et al., 2007; Dungait et al., 2012). Soil C storage in a mangrove ecosystem was also found primarily controlled by soil properties, especially the proportion of finer soil particles (Xiong et al., 2018). However, the type of chemical compounds may also be important, for example, recalcitrant organic C was reported to be a major fraction of SOC in an estuarine ecosystem of southern China (Lian et al., 2018). A high content in lignin- derived phenols and carbohydrates were found in mangrove soils (Marchand et al., 2005). Therefore, in addition to biogeochemical conditions, intrinsic SOC stabilization such as chemical protection may play a significant role in protecting SOC from decomposition.

In this study, we investigated the relative importance of SOC stabilization mechanisms in a coastal wetland, particularly between mangrove and salt marsh ecosystems. We assessed the mechanism of mineral association by comparing soil C fractions between particulate organic carbon (POC) and MOC, relative to soil silt/clay content. ^{13}C NMR spectroscopy was used to quantify the functional groups of SOC, which characterize SOC molecular structure. In addition, we investigated the microbial communities associated with each soil type by 16S rRNA and Internal Transcribed Spacer (ITS1) under different vegetation types. Specifically, we asked the following questions: (1) What is the relative importance of mineral association and chemical compounds in SOC stabilization between mangrove and salt marsh ecosystems? (2) How is microbial community involved in the SOC stabilization?

2. Materials and methods

2.1. Study site

This study was conducted in the subtropical Zhangjiang Estuary Mangrove National Nature Reserve (23°53′–23°57′N, 117°23′–117°30′E), Zhangzhou, Fujian Province in southeastern China. The mean annual temperature of this reserve is 22.2 °C and the annual mean rainfall was 1871.2 mm, most of which occurred from April to September. The tidal salinity ranges from 29 to 32 PSU. The reserve is characterized by dominant species of *Aegiceras corniculatum*, *Avicennia marina*, and *Kandelia obovata*, mixed with some other less common mangrove species such as *Bruguiera gymnorrhiza* and *Acanthus ilicifolius*. The exotic *S. alterniflora* has been widespread throughout the estuary since the late 1990s and this cordgrass is now a very common intertidal species, affecting the native species and adjacent ecosystem (Zhang et al., 2012). The soil classification of this area belongs to Solonchaks according to WRB (Spaargaren and Deckers, 1998).

2.2. Experimental design and soil sampling

We selected 3 transect lines that spanned the mangrove – salt marsh ecotone within the intertidal zone. Within each transect, we picked 3 sampling plots from each of the 3 communities: mangrove community, transitional community, and *S. alterniflora* community, so a total of 9 soil samples were collected in each transect. The sampling sites are shown in Fig. 1. 3 soil cores, 5–10 m apart, from each sampling plot were collected from 0 to 15 cm depth (a total of 27 soil cores) using a 15-cm-diameter auger in July 2017 and were transported to laboratory using a portable ice box. When performing NMR spectroscopy and microbial 16S rRNA analysis, 3 soil cores from same sampling plot were mixed to get homogenized soil.

2.3. Soil analysis

The soil samples for microbiological analysis were stored in −70 °C super cold refrigerator spare. The other soil samples were oven-dried at

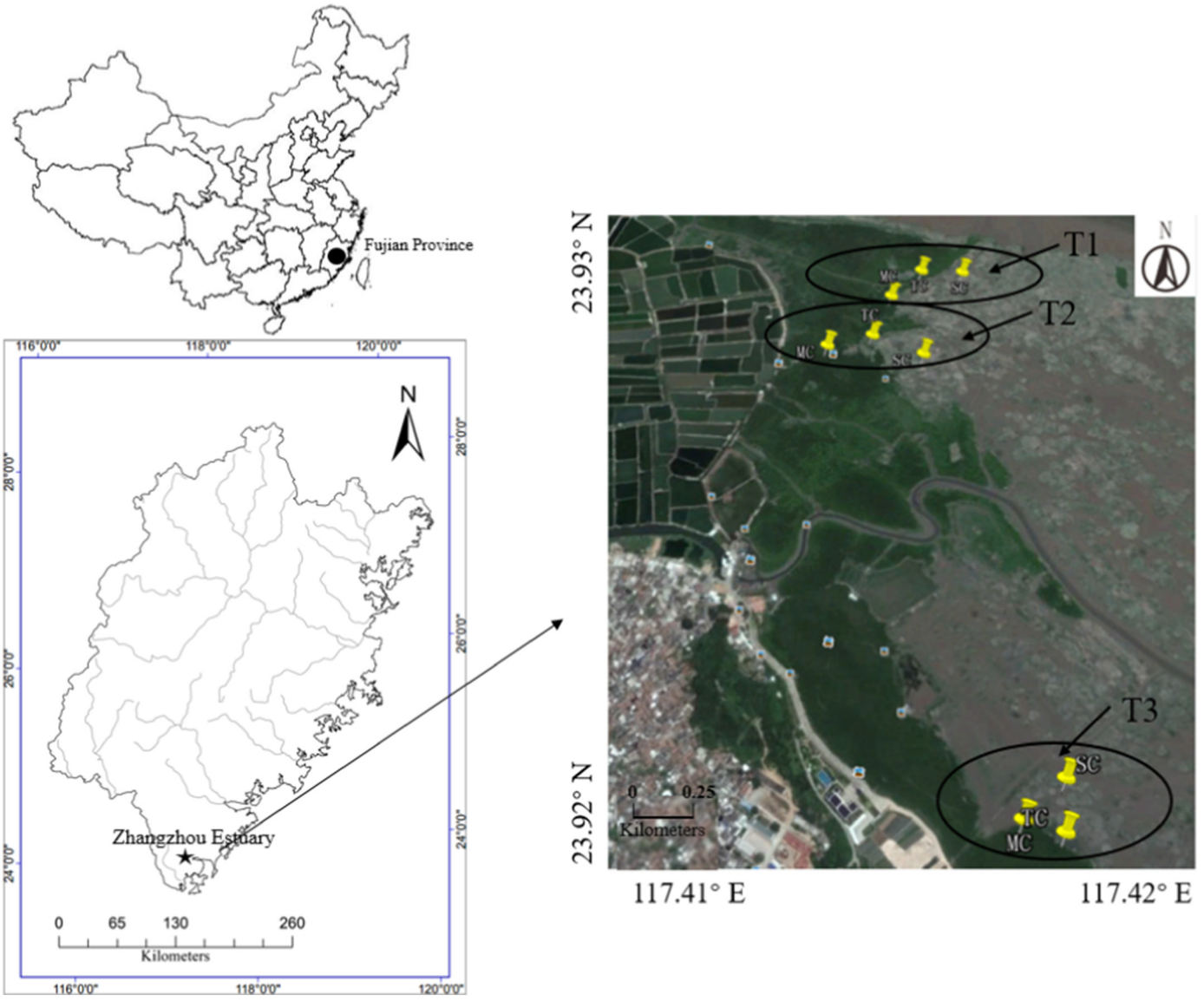

Fig. 1. Locations of the studied sites. The white circles in the right figure mean MC, TC and SC from landward to seaward. MC: mangrove community; TC: transitional community; SC: S. alterniflora community.

60 °C to constant weight, and weighed to determine soil water content. The roots in the soil were carefully picked out and then pass through a 2-mm sieve to remove plant residues and stones (Chen et al., 2018). The pH values of soil samples were determined by pH meter (PHS-3D, Rex, Shanghai, China). Air-dried subsamples were acidified with diluted HCl (5%) to remove carbonates, as described by (Nieuwenhuize et al., 1994). The procedure to extract fractionation of POC was modified from Cui et al. (2014). 20 g air-dried and 2-mm sieved soil was immersed in sodium hexametaphosphate (1 mol/L) for 24 h and then shaken at 180rev/min on a reciprocal shaker. Then, the soil was flushed with a continuous and steady water flow through the isolator onto a 53-μm sieve. The soil remaining on the 53-μm sieve, used to measure POC content, was dried to constant weight. We then took the extracted soil and air-dried soil to measure POC and SOC content, respectively, by elemental analyzer (Vario MACRO cube, Elementar, Langenselbold, Germany). SOC is total organic C in soil. Thus, MOC content was obtained by subtracting POC content from SOC content.

2.4. NMR spectroscopy

The soil samples used for NMR spectroscopy analysis were pretreated using HF(2%) to eliminate paramagnetic materials, e.g. ferric ion and manganese ion, that may affect the NMR signals (Skjemstad et al., 1994; Schmidt et al., 1997; Mathers et al., 2002). The solid-state NMR spectra (^{13}C-CP-MAS) were recorded on a Bruker AVANCE III 600 MHz instrument (Bruker Instrument Inc., Billerica, MA, USA). The acquisition conditions were set at frequency of 75.5 MHz, with 20 kHz spectra width, 5 kHz spinning speed, 2 ms contact time, and 2.5 s recycle time. The regions of 0–210 ppm spectra were plotted.

We examined the following seven chemical shift regions, representing the main C functional groups: alkyl C (0–45 ppm), N-alkyl C (45–60 ppm), O-alkyl C (60–90 ppm), di-O-alkyl C (90–110 ppm), Aromatic C (110–145 ppm), Phenolic C (145–165 ppm) and Carbonyl C (165–210 ppm). We quantified the percentage of each chemical shift as area under the curve of each chemical shift region divided by total area. For example, we used symbol C_{0-45} to

represent the percentage of 0–45 ppm region in the total region of 0–210 ppm, same as follows. We calculated 4 ratios that describe the characteristics of soil organic matter. The ratio of alkyl C to O-alkyl C (A/O-A = C_{0-45}/C_{60-90}) is an index represents the extent of SOC decomposition, the higher this ratio, the higher the decomposition degree of SOC. Aromaticity ($C_{110-165}/C_{0-165}$), was used to indicate the complexity of molecular structure. The ratio of Aliphatic C/aromatic C (Alip/Arom), $C_{0-110}/C_{110-165}$, also indicates the molecular structure of soil C, with higher Alip/Arom means less aromatic nuclear structure in humus. The Hydrophobic C/hydrophilic C (HB/HI) ratio, $(C_{0-45} + C_{110-165})/(C_{45-110} + C_{165-210})$, was used to reflect the stability of soil aggregation.

2.5. DNA extraction and polymerase chain reaction amplification

The composition of microbial communities including bacterial and fungi was analyzed by high-throughput sequencing, which was conducted by a professional biotechnology company, Annoroad Gene Technology Co., Ltd. Beijing, China. Here, we describe the procedure briefly. Total genome DNA from soil samples was extracted via a PowerSoil DNA extraction kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) using CTAB/SDS method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μl using sterile water. A fragment covering the V3 + V4 region of the 16S rDNA gene and a fragment covering the ITS1 region in the ITS rDNA gene were selected to construct bacterial and fungal community libraries. In the 16S rDNA gene region, the primer 341F (5'-CCTACGGG NGGCWGCAG-3′) and 805R (5'-GACTACHVGGGT ATCTAATCC-3′) amplify the V3 + V4 region, and the BITS (5'-ACC TGCGGARGGATCA-3′) and B58S3 (5'-GAGATCCRTTGYTRAAAGTT-3′) primers amplify in the ITS1 region. All polymerase chain reaction (PCR) reactions were carried out in 30 μL reactions with 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, and a final extension at 16 °C for 2 min. The same volume of 1× loading buffer (which contained SYBR green) was mixed with PCR products and electrophoresis was performed on 2% agarose gel for detection. Samples with bright main strip between 460 bp(V3 + V4)were chosen for further experiments. Samples with bright main strip between 100 and 300 bp (ITS1 hypervariable) were chosen for further experiments. PCR products were mixed in equidensity ratios. Then, the mixture PCR products were purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using NEB Next Ultra DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Life Technologies, CA, USA) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina HiSeq2500 using the 250 paired-end protocol. For post-sequencing quality filtering, we discarded the reads if the bar codes were uncorrectable, or the bases with a Phred Quality score < 19 covered over 50% of the read. The paired reads were spliced using PEAR (Zhang et al., 2013) software to merge sequences according to overlap of the clean data. Chimeric sequences were removed. Sequences were assigned to operational taxonomic units (OTUs) with 97% pairwise identity as the threshold, and then classified the representative sequence for each OUT taxonomically using the RDP classifier or Closed Reference anainst the Greengenes 16S rRNA reference database.

2.6. Statistical analysis

All data presented in the study were the mean values of three field replications. Any significant difference in soil physicochemical properties and percent of chemical shifts between different communities was determined by one-way ANOVA followed by Turkey's HSD post hoc test considering difference statistically at $p < 0.05$. Based on the OTU results, the phylum and class composition of bacteria and fungi were analyzed and the redundancy analysis (RDA) was performed with the Canoco 5 to examine the relationship between SOC fractions and top 10 most abundant bacteria and fungi at class level. To examine difference of microbial compositions among plant communities, we conducted permutation multivariate analysis of variance (PerMANOVA) test (Anderson, 2001), the difference was considered statistically at $p < 0.05$. Statistical analysis was conducted using SPSS 13.0.

3. Results

3.1. Soil organic carbon and soil properties

The sand content was significantly higher and silt/clay content was lower in mangroves than in transitional zones and *S. alterniflora*. Significant differences in pH were observed between the transitional community and *S. alterniflora*, and the EC were lower in *S. alterniflora* than the other two communities (Table 1). Soil C stock and other properties were presented in Table 1. SOC was highest in the soil of mangrove forest, followed by the transitional community and *S. alterniflora*, though there were no significant difference between mangroves and transitional zones. The majority of organic C in mangroves was POC, circa 90%, with a small percent of MOC, circa 10%. However, the percentage of MOC contents in the transitional zones (circa 55%) and *S. alterniflora* (circa 32%) were relatively higher than in the mangroves, corresponding to their higher silt and clay content.

3.2. Solid-state NMR spectroscopy

The NMR spectroscopy of soil samples exhibited different relative abundances of C functional groups among the three communities (Table 2). The alkyl C (0–45 ppm) and the O-alkyl C (60–90 ppm) showed highest peaks in all soil samples, followed by aromatic C (110–145 ppm) and carbonyl C (165–210 ppm). Detail graphs of the spectra can be found in our previous study at (Sun et al., 2018). Soils from the transitional community had lower alkyl C (0–45 ppm) and higher O-alkyl C (60–90 ppm) than both from mangrove and *S. alterniflora* communities, which resulted in the lowest ratio of alkyl C to O-alkyl C (A/O-A). The aromaticity in *S. alterniflora*-vegetated soil was significantly lower than in mangrove and the transitional community, and Alip/Arom ratio was significantly higher in *S. alterniflora* communities than in transitional communities and mangroves, indicating that less aromatic nuclear structure in humus and simpler molecular structure in soils of *S. alterniflora* communities. The Hydrophobic C/hydrophilic C (HB/HI) ratio was not significantly different among the three vegetation types.

3.3. Composition of microbial communities

In the bacterial community, a total of 21,008 OTUs were obtained at equal sequencing depth, with 97% sequence similarity. According to the

Table 1
Soil properties (mean ± SD, n = 9) from different plant communities. Different letters in a single column indicate a significant difference between the plant communities ($p < 0.05$).

Plant community	Sand(%)	Silt+/clay(%)	Water content (g/g)	pH	EC(mS·cm-1)	SOC(g/kg)	POC(g/kg)	MOC(g/kg)
Mangrove	15.98 ± 4.34a	84.02 ± 4.41a	0.95 ± 0.15a	5.69 ± 0.78ab	2.52 ± 0.63a	16.49 ± 4.96a	14.85 ± 1.6a	1.64 ± 0.26a
Transitional community	6.31 ± 2.49b	93.69 ± 3.02b	1.10 ± 0.24a	5.15 ± 0.84a	2.03 ± 0.69a	15.85 ± 2.81a	7.11 ± 0.26b	8.74 ± 1.27b
S. alterniflora	3.33 ± 0.84b	96.67 ± 1.81b	0.90 ± 0.21a	6.18 ± 0.44b	1.45 ± 0.29b	10.23 ± 2.33b	6.97 ± 0.44b	3.26 ± 0.63a

Table 2
Relative intensities (mean ± SD, n = 3) of different carbon chemical shifts from solid-state ^{13}C CPMAS NMR spectra of soil samples from different plant communities. A/O-A = Alkyl C/O-alkyl C; Alip/Arom = Aliphatic C/aromatic C; HB/HI = Hydrophobic C/hydrophilic C. Different letters in a single row indicate a significant difference between the plant communities ($p < 0.05$).

Chemical shifts		Mangrove	Transitional community	*S. alterniflora*
^{13}C NMR (%)	Alkyl C (0–45 ppm)	19.96 ± 1.86ab	15.72 ± 0.93a	24.03 ± 3.17b
	N-alkyl C (45-60 ppm)	7.29 ± 0.23a	8 ± 0.24a	8.37 ± 1.1a
	O-alkyl C (60–90 ppm)	21.65 ± 1.98a	26 ± 1.3a	23.49 ± 5.12a
	di-O-alkyl C (90–110 ppm)	10.79 ± 0.71ab	11.85 ± 0.26a	9.58 ± 1.67b
	Aromatic C (110-145 ppm)	17.23 ± 0.71a	18.58 ± 0.38b	15.75 ± 0.29c
	Phenolic C (145-165 ppm)	9.11 ± 0.4a	8.41 ± 0.42a	6.56 ± 1.02b
	carbonyl C (165–210 ppm)	13.97 ± 2.39a	11.43 ± 0.94a	12.23 ± 4.75a
	A/O-A (C_{0-45}/C_{60-90})	0.5 ± 0.05ab	0.34 ± 0.03a	0.6 ± 0.19b
	Aromaticity ($C_{110-165}/C_{0-165}$)	0.31 ± 0.02a	0.3 ± 0.01a	0.25 ± 0.02b
	Alip/Arom ($C_{0-110}/C_{110-165}$)	2.27 ± 0.19a	2.28 ± 0.07a	2.94 ± 0.29b
	HB/HI ($\frac{C_{0-45}+C_{110-165}}{C_{45-110}+C_{165-210}}$)	0.86 ± 0.04a	0.75 ± 0.03a	0.87 ± 0.1a

results of perMANOVA analysis, there was no significant difference in microbial composition between plant communities (bacteria: $p = 0.183$; fungi: $p = 0.458$). The relative abundance of each component at phylum and class level for bacterial and fungi were exhibited in Figs. 2 and 3, respectively. At the phylum level, the bacterial community of soil samples were dominated by *Proteobacteria* (circa 55%), followed by *Bacteroidetes* (circa 9.8%), *Acidobacteria* (circa 4%), *Chloroflexi* (circa 3.4%), and *Nitrospirae* (circa 3%) across all the communities (Fig. 2a). The samples collected from the mangrove forest had a greater abundance of *Bacteroidetes* (circa 12.6%) but fewer *Nitrospirae* (circa 2.2%). At the class level (Fig. 2b), the microbial taxa detected in our samples pointed to a predominance of sulfate reducers, characterized by the *Gammaproteobacteria* (circa 19.7%) and *Deltaproteobacteria* (circa 19.2%). *Deltaproteobacteria* was more abundant than *Gammaproteobacteria* in mangrove soils and transitional communities, but the opposite was true in *S. alterniflora* communities. Soil samples collected from transitional communities had higher *Epsilonproteobacteria* than mangroves and *S. alterniflora* communities.

In the fungi community (Fig. 3), a total of 2964 OTUs were obtained by ITS sequencing from 9 soil samples in three vegetation types. The OTU richness followed the pattern of Mangrove Community> Transitional Community> *S. alterniflora* Community with no significant difference. Nearly 37% of these fungi belonged to the *Ascomycota*, following by *Basidiomycota* (5.6%). The *Zygomycota* (circa 0.05%) were only found in *S. alterniflora* communities. At the class level (Fig. 3b), *Sordariomycetes* (circa 11.77%) and *Dothideomycetes* (circa 11.42%) were dominant in soils from three communities. The quantities of remaining identified fungus were relatively low (circa 7%) and unclassified fungus make up a great proportion (circa 70%).

3.4. Relationship between C functional groups and microbial communities

The redundancy analysis (RDA) was applied to assess the association of C chemical shifts and microbial communities at the class level. The relationship between bacterial community and C chemical shifts was shown in Fig. 4 (axis 1 = 74.16%, axis 2 = 22.35%) and the association of chemical shifts and fungi community was shown in Fig. 5 (axis 1 = 74.23%, axis 2 = 22.64%). In the bacteria community, the results indicated that the *Gammaproteobacteria*, which dominated the soil samples in three vegetation types, were positively associated with Alkyl C and A/

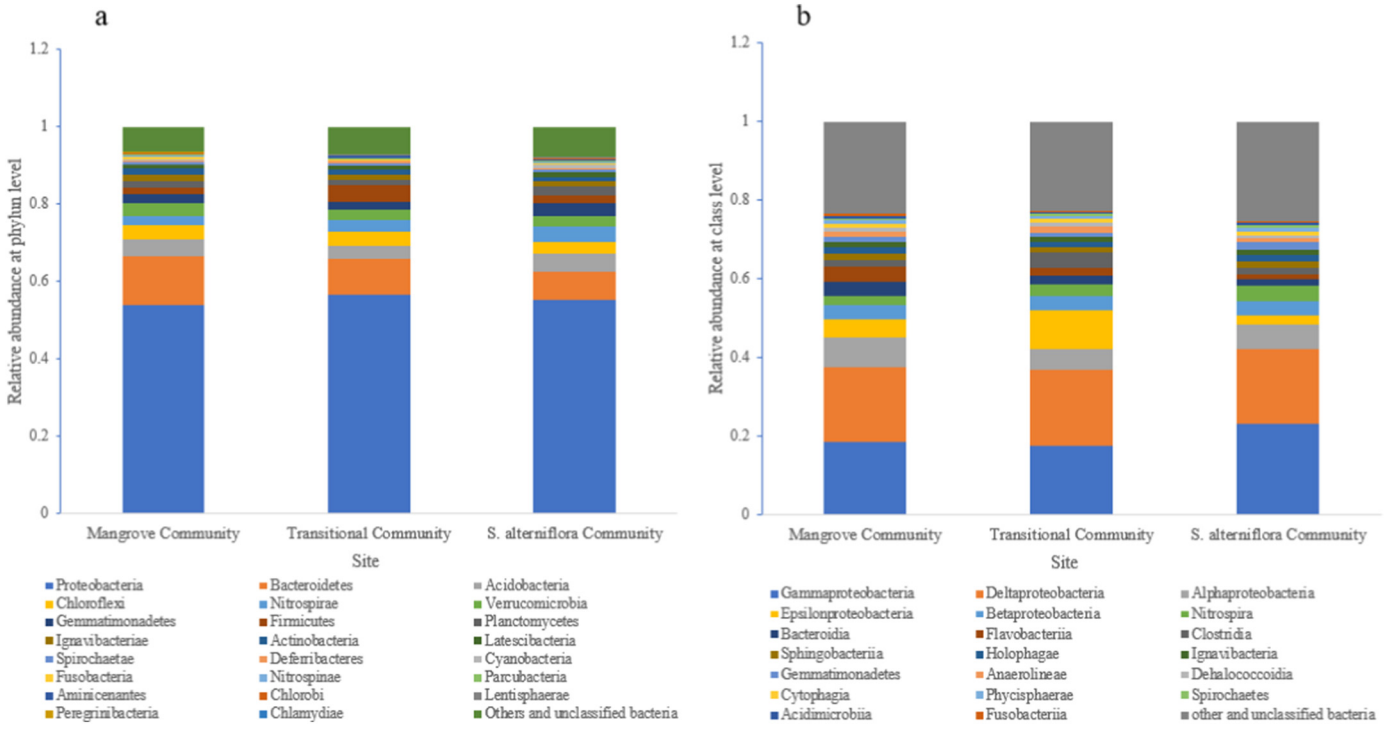

Fig. 2. Relative abundance of bacterial community at phylum level (a) and at class level (b).

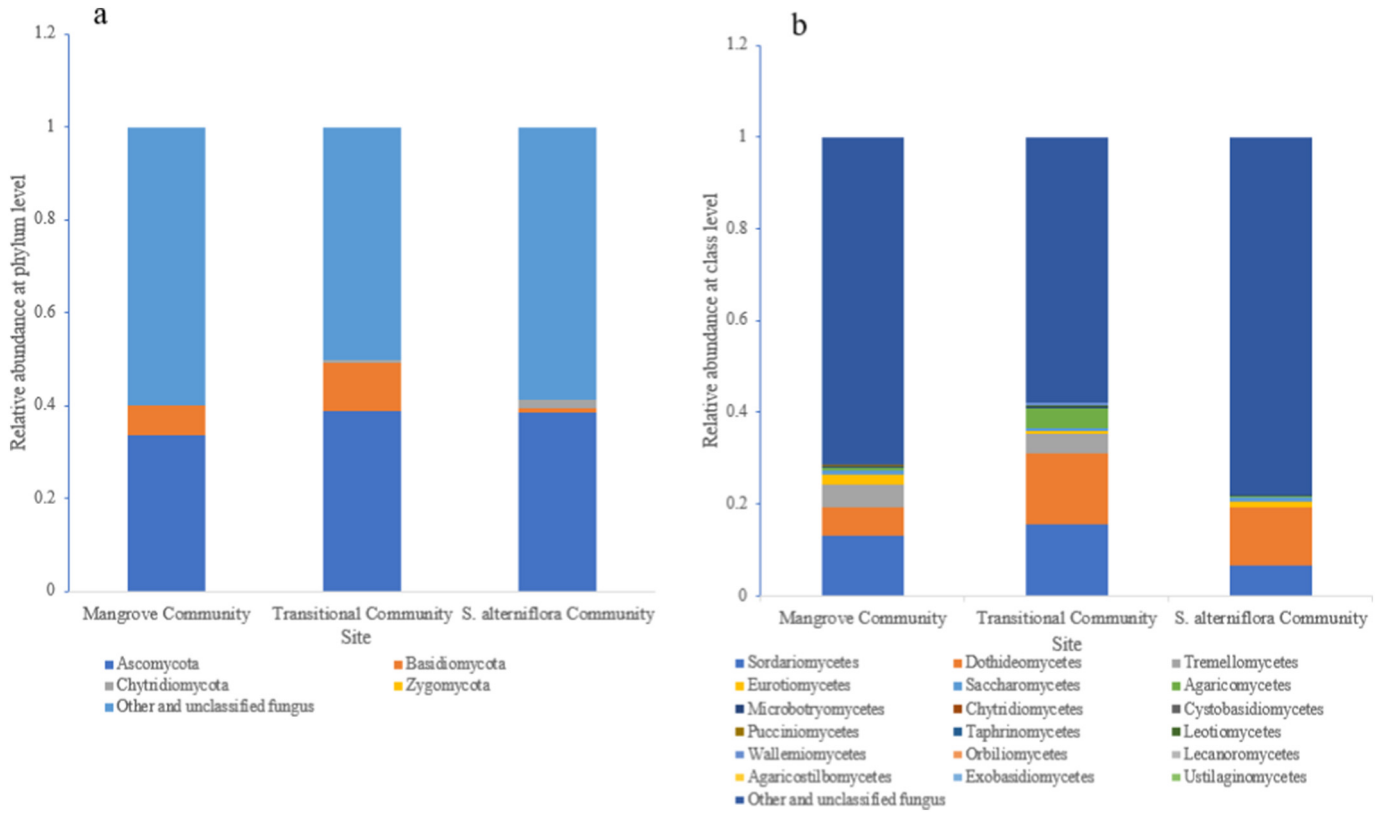

Fig. 3. Relative abundance of fungi community at phylum level (a) and at class level (b).

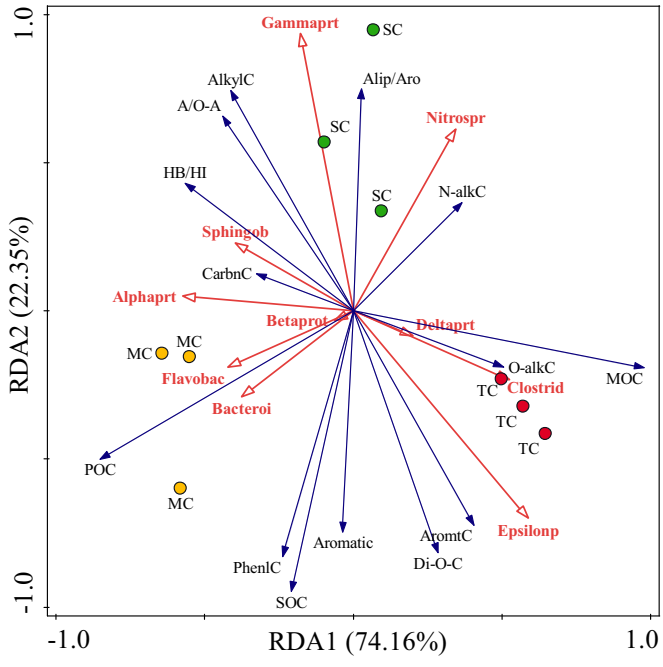

Fig. 4. Redundancy analysis (RDA) correlation plot showing variance in bacteria community composition of class explained by C forms. Note: Deltaprt = *Deltaproteobacteria*; Gammaprt = *Gammaproteobacteria*; Epsilonp = *Epsilonproteobacteria*; Alphaprt = *Alphaproteobacteria*; Nitrospr = *Nitrospira*; Betaprot = *Betaproteobacteria*; Bacteroi = *Bacteroidia*; Flavobac = *Flavobacteriia*; Clostrid = *Clostridia*; Sphingob = *Sphingobacteriia*; N-alkC=N-alkyl C; O-alkC = O-alkyl C; Di-O-C = di-O-alkyl C; AromtC = Aromatic C; PhenlC = Phenolic C; Aromatic = Aromaticity; A/O-A = Alkyl C/ O-alkyl C; Alip/Aro = Aliphatic C/aromatic C; HB/HI = Hydrophobic C/hydrophilic C.

O-A. O-alkyl C and MOC were positively associated with *Deltaproteobacteria* and *Clostridia*, while POC was associated with *Flavobacteriia* and *Bacteroidia*. In the fungi community, *Pucciniomycetes*, *Microbotryomycetes*, *Tremellomycetes* and *Sordariomycetes* all showed positive correlation with SOC and aromaticity. *Dothideomycetes* was associated with MOC and O-alkyl C.

4. Discussion

In this paper, the relative importance of SOC stabilization mechanisms in coastal wetland was studied (Lehmann et al., 2007; Cui et al., 2014; Sarker et al., 2018). Our experiment, based on different soil samples from mangrove/salt marsh transitions, found that soil C storage and chemical composition of C functional groups were significantly different among different plant communities. Our results provided a novel contribution towards the understanding of the differences in the soil C stability mechanisms among mangroves and salt marshes.

4.1. Mineral protection of SOC in mangrove and salt marsh ecosystems

Consistent with most of previous studies, our results confirmed that SOC content was highest in mangrove forest, followed by the transitional community and *S. alterniflora* (Bai et al., 2017). Chmura et al. (2003) compiled data for 154 sites in mangroves and salt marshes on a global scale and found that the average soil C density of mangroves (0.055 ± 0.004 g/cm^3) is significantly higher than the salt marsh (0.039 ± 0.003 g/cm^3). Accumulation of SOC is usually due to input from plant litter and output from C mineralization. Although we did not measure plant litter input and C efflux directly, the majority of SOC in mangrove forest was POC, indicating high plant litter input to soil may contribute the higher SOC in mangrove forest. In a study at the Caribbean region, McKee (2011) found that root production contributed to the soil accretion in mangrove forest. Our results indicated that lower soil pH in the mangrove forest and transitional community than

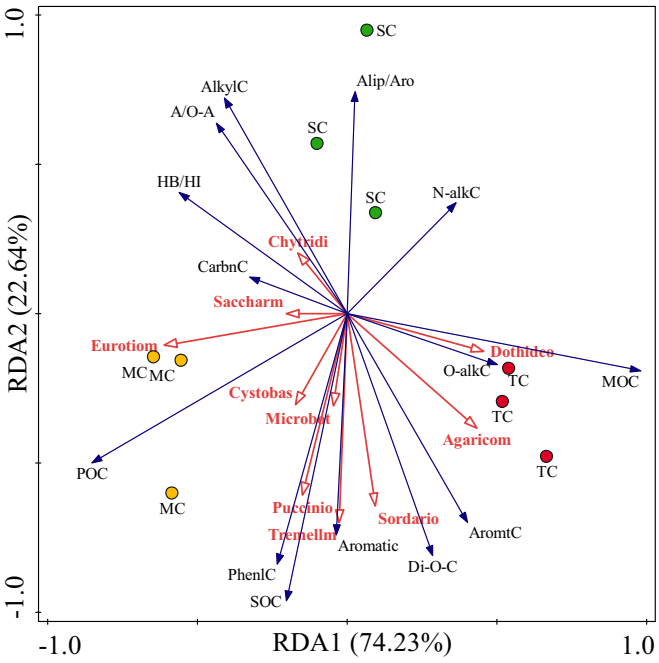

Fig. 5. Redundancy analysis (RDA) correlation plot showing variance in fungi community composition of class explained by C forms. Note: Dothideo = *Dothideomycetes*; Sordario = *Sordariomycetes*; Eurotiom = *Eurotiomycetes*; Tremellm = *Tremellomycetes*; Agaricom = *Agaricomycetes*; Microbot = *Microbotryomycetes*; Saccharm = *Saccharomycetes*; Chytridi = *Chytridiomycetes*; Puccinio = *Pucciniomycetes*; Cystobas = *Cystobasidiomycetes*; N-alkC=N-alkyl C; O-alkC=O-alkyl C; Di-O-C = di-O-alkyl C; AromtC = Aromatic C; PhenlC=Phenolic C; Aromatic = Aromaticity; A/O-A = Alkyl C/ O-alkyl C; Alip/Aro = Aliphatic C/aromatic C; HB/HI = Hydrophobic C/hydrophilic C.

in *S. alterniflora* may also contribute to the SOC content. Beets et al. (2002) clarified significant negative relationships between soil C and pH, pointing out that Al/humic acid complexes increased under lower soil pH and, thereby, accelerated the accumulation of soil C. However, further studies are needed to investigate the relative contribution of plant litter input and C efflux on the accumulation of SOC in mangrove and salt marsh ecosystem.

We further found that the percentages of MOC in transitional community (circa 55%) and *S. alterniflora* (circa 32%) were much higher than in mangrove forest (circa 10%). This is probably due to high silt/clay content in transitional zones and *S. alterniflora*-vegetated area, which contributes to the mineral protection of SOC. High O-alkyl C from the transitional community, usually derived from labile C, confirmed the mechanism that either aggregation protection or mineral association may be playing a role inhibiting SOC decomposition. Nevertheless, aggregation was reported to be less important in wetland soils (Cui et al., 2014), pointing to the mineral association as a main mechanism of SOC protection in the transitional zone (Six et al., 2002). Highest silt/clay content in *S. alterniflora* communities did not result in highest MOC percentage. This is probably due to the high pH in *S. alterniflora*-vegetated soil promoting desorption of SOC with mineral surface (Liu and Gonzalez, 1999).

4.2. Recalcitrance of SOC in mangrove and salt marsh ecosystems

Our results from the ^{13}C CPMAS NMR spectroscopy assessing the relative intensities of different C functional groups were within the values usually found in soils (Mahieu et al., 1999), with the intensity of the O-alkyl C spectral region being the highest in mangrove and transitional zone expect for the spartina community in which the soil has most abundant alkyl C (Table 2). A high amount of O-alkyl C indicates soil C with a high potential for decomposition (Kavdır et al., 2005). The anaerobic conditions in coastal wetland could have reduced the rate of organic matter decomposition and therefore resulted in the accumulation of relatively fresh organic matter (Canavan et al., 2006). But much lower A/O-A ratio in transitional community should result from other mechanisms such as mineral association.

The A/O-A ratio has been used to indicate the extent of decomposition of organic matter (Baldock and Preston, 1995). In our study site, soil samples from *S. alterniflora* were highly decomposed, although high silt/clay content should have protected them from decomposition. The microbial residual at late stage of decomposition was usually recalcitrant (Voroney et al., 1989), which prevented SOC from further decomposition in *S. alterniflora* vegetated soil. However, in mangrove forest, SOC stabilization was mainly controlled by recalcitrance of aromatic C. There was no significant difference of aromatic C between mangrove forest and the transitional community, indicating that *S. alterniflora* invasion at the transitional zone (Wang et al., 2016; Bai et al., 2017) did not change aromatic C originating from mangrove trees. Analogously, a higher ratio of Alip/Arom means less aromatic nuclear structure in humus and simpler molecular structure of soil C. The ratio was highest in *S. alterniflora* and there was no significant difference between mangroves and transitional zones, consistent with the aromaticity. Together with highest Alkyl C in *S. alterniflora* suggests that after the encroachment of *S. alterniflora*, the molecular structure will be simpler and easier to be decomposed.

The HB/HI ratio can reflect the stability of the combination of SOC and aggregation. The larger the ratio, the higher the stability of SOC caused by soil aggregation (Spaccini et al., 2006). We did not measure soil aggregation experimentally, because the high salt content in coastal wetland soils makes the soil hard and tough after drying, which could further change the soil aggregation fractionation (Cui et al., 2014). Here in this study, the HB/HI showed no significant difference between the soils of different plant communities, indicating that the aggregation dynamics of the soils are likely to be similar. Moreover, it was found that O-alkyl and di-O-alkyl C fractions were positively associated with aggregate stability at medium-to-long term, indicating a promoting effect on soil structure (Sarker et al., 2018). Accordingly, Kavdır et al. (2005) found that the carbohydrate content of organic amendments was positively correlated to the stability of soil aggregates. Also, no significant differences were found in O-alkyl and di-O-alkyl C in mangroves and *S. alterniflora*, suggesting there were no difference in the aggregate stability between the mangroves and *S. alterniflora*.

4.3. Microbial community in soils of mangrove and salt marsh ecosystems

Vegetation types shaped the relative abundance and composition of microbial communities according to the results of composition of microbial communities. The result of RDA analysis found that SOC forms and chemical composition could explain a high proportion of the variation of soil microbial abundance. For example, the amount of *Proteobacterial* was positively correlated with MOC and N-alkyl C. Consistent with our results, the *Proteobacteria* were found to be dominant in bacterial communities in a Taiwan mangrove forest (Liao et al., 2007). The *Proteobacterial* play a significant role in the soil nutrient cycle, including the fixation of nitrogen (Holguin et al., 2001), which was associated with MOC and N-alkyl in our study, indicating that the amount of *Proteobacterial* has a positively correlated with soil mineral stabilization. These results suggest that the microbial community structure and abundance is likely to be a productive line of research and should be taken into consideration when investigating the properties of soil C and stability mechanism, rather than being studied, isolated or neglected.

5. Conclusions

In summary, our result indicated that the invasion of *S. alterniflora* into mangrove can result in an increase of SOC decomposition, which hindered the accumulation of SOC. We found that organo-mineral

association was the major mechanism of SOC stabilization in salt marshes, similar to most of other terrestrial ecosystems. However, recalcitrant compounds, as indicated by relatively high aromaticity and low MOC, mainly controlled the SOC stabilization in the mangroves. The SOC molecular structure of *S. alterniflora* soils was simpler, with significantly higher A/O-A and lower aromaticity, indicating a high degree of decomposition. Besides, the differences in SOC forms and chemical composition could explain a high proportion of the variation of soil microbial abundance.

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